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Fat synthesis in adipose tissue

An examination of stoichiometric constraints

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The requirement for net balance of synthesis, degradation and transport for all intermediates in the pathways from glucose to fat imposes constraints on the balance of fluxes between different pathways. Linear programming has been used to examine the interactions between these constraints on metabolism in adipocytes and the requirement for efficiency in the conversion of glucose into fat. The circumstances under which excessive ATP synthesis would accompany this conversion have been investigated.

INTRODUCTION

The existence of a metabolic steady state imposes strong constraints on the interactions between metabolic pathways, whatever the particular mechanisms by which they are regulated. Each metabolite is constrained by the requirement that its rate of appearance (through synthesis or transport) must balance its rate of disappearance in each cellular compartment. Simple instances of the effects of these stoichiometric constraints on metabolism are well known; for example, cytoplasmic pyridine-nucleotide balance constrains fermentations. In the study of the carbohydrate and fat metabolism of adipose tissue, there have been several experimental approaches to determination of the balance of carbon flux, $NAD^+/NADH$, $NADP^+/NADPH$, adenine nucleotides and cytoplasmic–mitochondrial exchanges (Katz & Rognstad, 1966; Flatt, 1970; Martin & Denton, 1970). Generally, a pattern of flows consistent with steady state has been obtained inductively from experimental measurements of accessible fluxes or enzyme activities. Stoichiometric constraints have not been built into the analysis of isotopic fluxes in this system as they were in the work of Rabkin & Blum (1985) on liver gluconeogenesis. Nevertheless, it should be possible to deduce some of the general consequences of stoichiometric constraints in this system, for the metabolic capabilities of adipose tissue are limited to the support of a small range of principal functions. However, such a system has reached a size where the determination of the relative fluxes required to achieve overall balance is not easy by hand. Watson (1984, 1986) has shown that such problems can be tackled by the numerical technique of linear programming. This selects the particular solution that minimizes some cost (such as utilization of an oxidizable substrate) or maximizes some yield (such as triacylglycerol formed) from amongst the possible solutions that are consistent with the requirements of balance. Although it is not certain that a cell can regulate its metabolism to achieve such optimal solutions, they represent possible end points of adaptation to evolutionary pressure and thus constitute interesting reference points. [A possible instance of metabolic optimization in evolution is given by Melendez-Hevia & Isidoro (1985), who have used a game-theory approach to demonstrate that the reactions of the pentose cycle are an optimal solution to the problem of rearranging pentoses.]

Here we use linear programming to examine the nature of the constraints on the synthesis of triacylglycerol from glucose in rat adipose tissue and to demonstrate the patterns of metabolism that optimally satisfy various goals.

THE MODEL

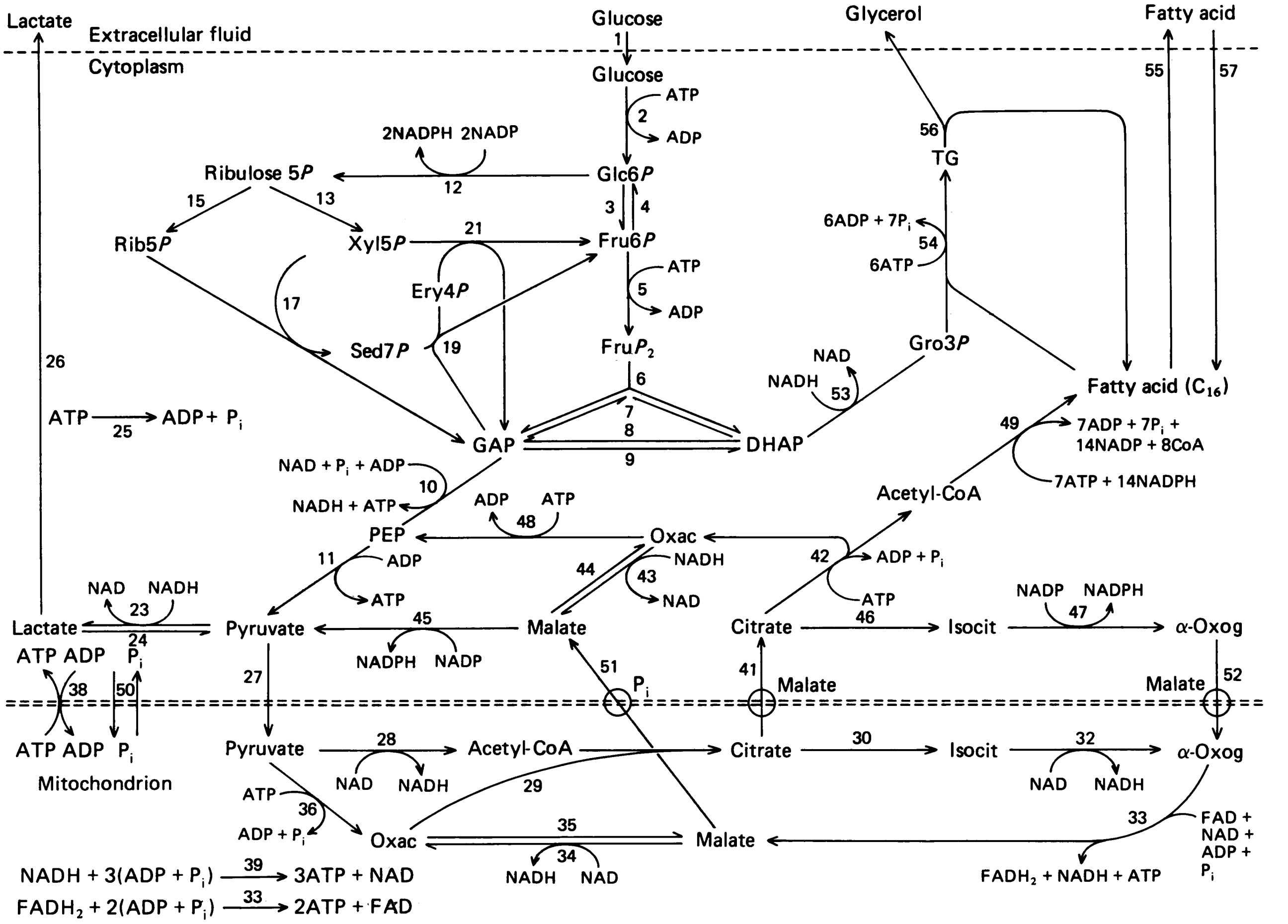
The model (Scheme 1) includes the major metabolic pathways and the suggested shuttle systems between cytoplasm and mitochondria in adipose tissue (Martin & Denton, 1970), although no mechanism has been provided for mitochondrial oxidation of cytoplasmic NADH. There are over 50 reactions and intermediates, even after condensation of some of the processes into a single overall reaction. The triacylglycerol (TG) is represented as tripalmitoylglycerol. The exchange systems between mitochondria and cytoplasm are assumed to be perfectly coupled, and citrate is assumed to be the only form in which two-carbon units can be exported from the mitochondrion. The P/O ratio is assumed to be the same inside and outside the mitochondria, i.e. no allowance has been made for a difference in the effective stoichiometry that may be caused by energy linkage of ATP/ADP exchange (Azzone *et al.*, 1984).

For presentation as a problem in linear programming, the model is represented as:

$$
\begin{array}{c}
a_{11}x_1 + a_{12}x_2 + \cdots a_{1n}x_n = \text{or} > \text{ or } < b_1 \\
a_{21}x_1 + a_{22}x_2 + \cdots a_{2n}x_n = \text{or} > \text{ or } < b_2 \\
\vdots \qquad\qquad \vdots \\
a_{m1}x_1 + a_{m2}x_2 + \cdots a_{mn}x_n = \text{or} > \text{ or } < b_m
\end{array}
$$

where m is the number of chemicals in the model; n is the number of reactions in the model; a_{ij} is the stoichiometric coefficient of the ith chemical in the jth reaction, negative

Abbreviation used: TG, triacylglycerol.



Scheme 1. Reactions of the model of adipocyte metabolism

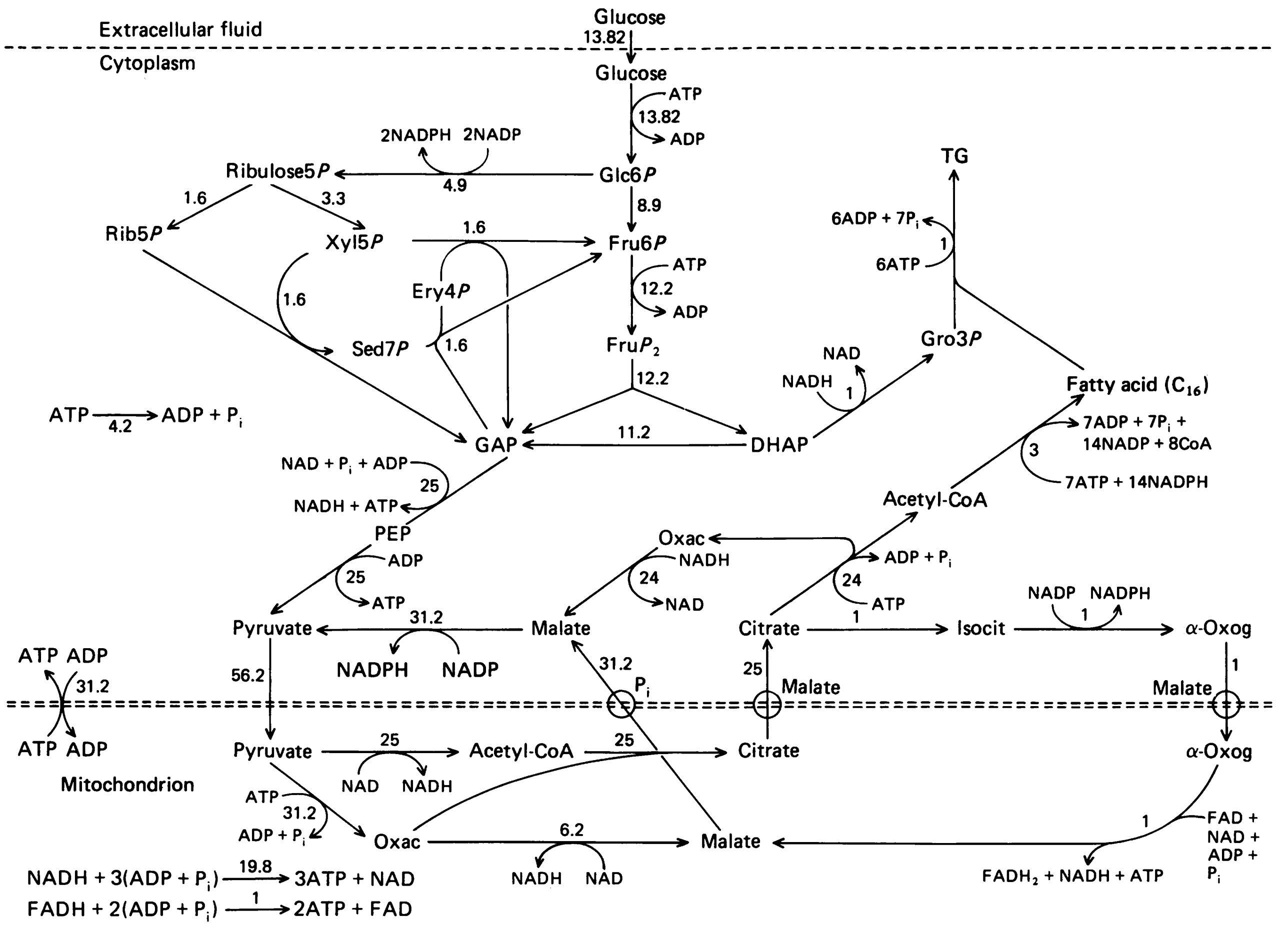
The numbers in the Scheme refer to the enzymes catalysing the reactions as follows: 1, glucose transport; 2, hexokinase (2.7.1.1); 3 and 4, glucose-6-phosphate isomerase (5.3.1.9); 5, 6-phosphofructokinase (2.7.1.11); 6 and 7, aldolase (4.1.2.13); 8 and 9, triosephosphate isomerase (5.3.1.1); 10, glyceraldehyde-phosphate dehydrogenase (1.2.1.12)+phosphoglycerate kinase (2.7.2.3)+phosphoglycerate mutase (5.4.2.1)+enolase (4.2.1.11); 11, pyruvate kinase (2.7.1.40); 12, glucose-6-phosphate dehydrogenase (1.1.1.49)+6-phosphogluconolactonase (3.1.1.31); 13, ribulose-phosphate 3-epimerase (5.1.3.1); 15, ribose-5-phosphate isomerase (5.3.1.6); 17 and 21, transketolase (2.2.1.1); 19, transaldolase (2.2.1.2); 23 and 24, lactate dehydrogenase (1.1.1.27); 25, unspecified ATP utilization reactions; 26, lactate transport; 27, pyruvate transport; 28, pyruvate dehydrogenase complex (1.2.4.1+2.3.1.12+1.8.1.4); 29, citrate synthase (4.1.3.7); 30, aconitate hydratase (mitochondrial) (4.2.1.3); 32, isocitrate dehydrogenase (NAD^+) (mitochondrial) (1.1.1.41); 33, tricarboxylic acid cycle from α-oxoglutarate to malate; 34 and 35, malate dehydrogenase (mitochondrial) (1.1.1.37); 36, pyruvate carboxylase (6.4.1.1); 38, ATP ⇀↽ ADP exchange; 39, electron transport and oxidative phosphorylation from NADH to O_2; 40, electron transport and oxidative phosphorylation from $FADH_2$ to O_2; 41, citrate ⇀↽ malate exchange; 42. ATP citrate (*pro*-3*S*)-lyase (4.1.3.8); 43 and 44, malate dehydrogenase (cytoplasmic) (1.1.1.37); 45, malate dehydrogenase (decarboxylating) ($NADP^+$) (1.1.1.40); 46, aconitate hydratase (cytoplasmic) (4.2.1.3); 47, isocitrate dehydrogenase ($NADP^+$) (cytoplasmic) (1.1.1.42); 48, phosphoenolpyruvate carboxykinase (GTP) (4.1.1.32); 49, fatty-acid-synthesis pathway; 50, phosphate transport; 51, malate ⇀↽ phosphate exchange; 52, malate ⇀↽ α-oxoglutarate exchange; 53, glycerol-3-phosphate dehydrogenase (NAD^+) (1.1.1.8); 54, TG synthesis; 55, fatty acid export; 56, hormone-sensitive lipase (3.1.1.3); 57, fatty acid import. The enzyme catalogue numbers (IUB, 1984) are given in parentheses. Further abbreviations used: Ribulose5*P*, ribulose 5-phosphate; Glc6*P*, glucose 6-phosphate; Rib5*P*, ribose 5-phosphate; Xyl5*P*, xylose 5-phosphate; Fru6*P*, fructose 6-phosphate; Ery4*P*, erythrose 4-phosphate; Sed7*P*, sedoheptulose 7-phosphate; FruP_2, fructose bisphosphate; Gro3*P*, glycerol 3-phosphate; GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate, PEP, phosphoenolpyruvate; Oxac, oxaloacetate; Isocit, isocitrate; α-Oxog, α-oxoglutarate.

where the chemical is consumed, positive where it is a product, and 0 where it is not involved in the reaction; the $m \times n$ matrix of a_{ij} is the stoichiometry matrix; x_j is the relative flux through the jth reaction. Since all x values are required to be zero or positive, any reversible reaction has to be entered as separate forward and reverse reactions; b_i comprises, with the preceding sign ($=$, $<$ or $>$) the constraint on the ith chemical. For the internal metabolites, the constraint is equal to zero, i.e. there is no net synthesis or degradation. Source chemicals are set to a negative value or allowed to be < 0; products are set to a positive value or allowed to be > 0.

The solution required is the vector of relative fluxes, x, that minimizes a cost function:

$$f(x) = c_1x_1 + c_2x_2 \cdots\cdots + c_nx_n$$

Most of the c_i values are zero in this problem; a reaction that is to be minimized (e.g. use of external glucose) is



Scheme 2. Relative fluxes achieving maximum economy of glucose utilization during TG synthesis

The numbers in the Scheme give the calculated relative fluxes through the reactions. Eqn. (1) in the text summarizes this result. Note that malate and phosphate balances include their involvement in exchange mechanisms at the mitochondrial membrane. For abbreviations, see Scheme 1.

given a positive coefficient, c, and a reaction that is to be maximized (e.g. yield of TG) can be given a negative cost coefficient.

A problem cast in this standard form possesses a number of favourable features that aid the search for a solution. The principles of strategies available for obtaining numerical solutions can be found in texts on linear programming (e.g. Bunday, 1984). The method used here was the Revised Simplex method, implemented for the computer by NAG as routine H01ADF (NAG, 1984). This was called from a PASCAL program that sets up appropriate cost and constraint vectors and links the non-zero elements of the solution vector with the appropriate reaction for output. The program runs on PRIME computers under the PRIMOS operating system, or on a Ferranti PC860 under MS-DOS 2.11. The large stoichiometry matrices were obtained as output from the reaction-parsing unit of a metabolic simulation and control analysis package SCAMP (Sauro, 1986), which takes the reactions as input in text form.

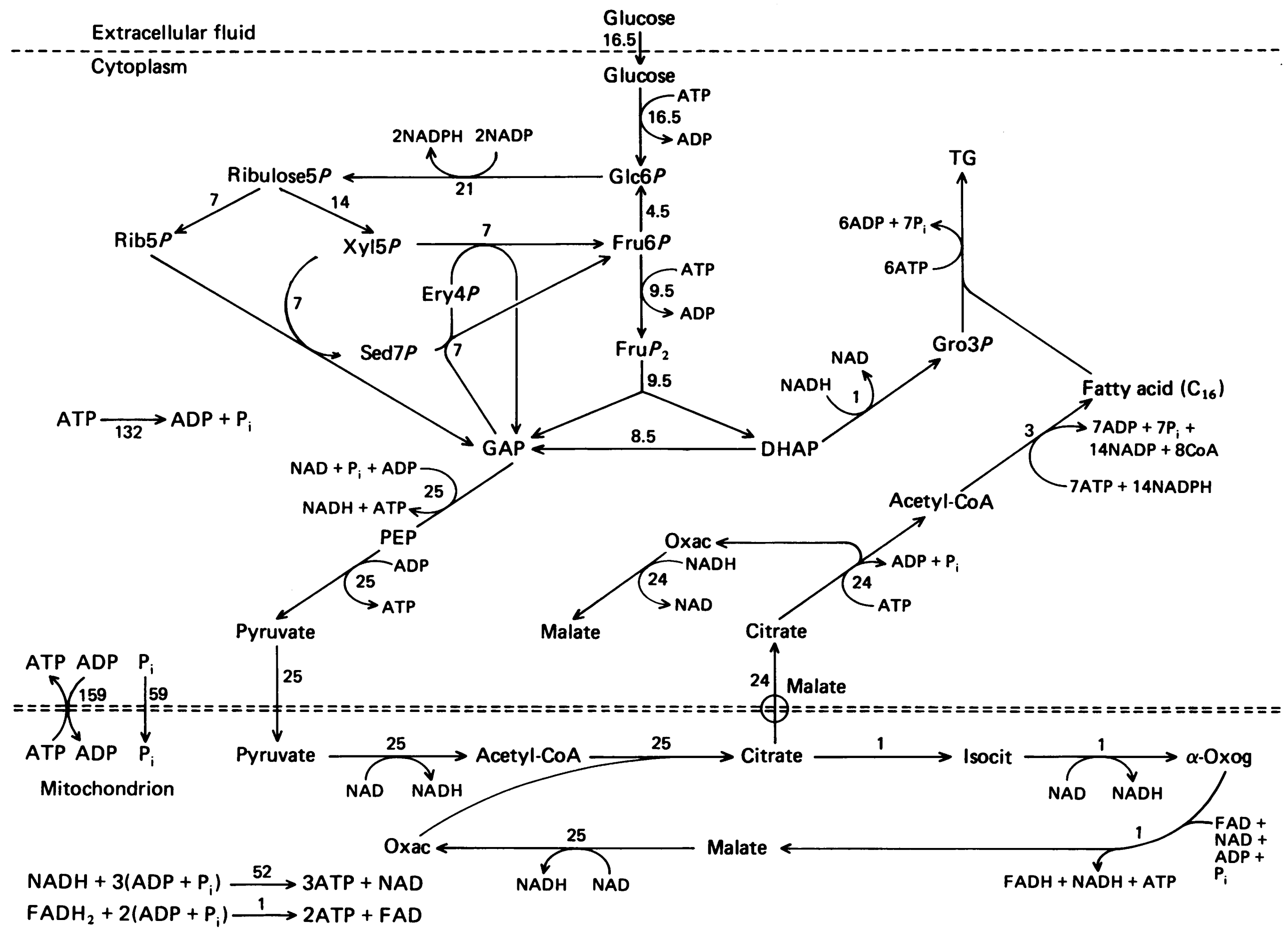
Metabolites in different compartments are treated as different chemicals, and transport processes between compartments are written as reactions. In addition, 'dummy metabolites' were written into certain reactions to allow constraints to be placed on those reactions.

RESULTS

The simplest example of the model is the utilization of glucose to produce TG, with no hydrolysis of stored TG or utilization of external fatty acids. It was not initially possible to obtain any solution of this problem without allowing excess ATP production to occur, which would have to be balanced by ATP consumption in the cell by processes not specified in the model. Different solutions were obtained with different cost functions. Thus minimizing the amount of glucose used per TG formed gives the reaction pattern shown in Scheme 2, and the overall stoichiometry:

$$13.82\,\text{Glucose} + 4.2(\text{ADP} + \text{P}_i) \rightarrow 1\,\text{TG} + 31.92\,\text{CO}_2 + 4.2\,\text{ATP} \quad (1)$$

Flatt (1970) noted that the conversion of glucose into TG would result in 1 ATP per acetyl group and a glucose utilization of 14.1 per TG, but our values are lower (eqn. 1 and Table 1) In this case, much of the NADPH required for fatty acid synthesis comes from malate dehydrogenase (decarboxylating). If the generation of NADPH from the pentose pathway is maximized (Scheme 3), and that from 'malic' enzyme minimized, then hexose units are recycled through the pathway, and



Scheme 3. Relative fluxes maximizing production of NADPH in the pentose pathway

The numbers in the Scheme give the calculated relative fluxes through the reactions. Eqn. (2) in the text summarizes this result. Note that malate balance involves its exchange for citrate at the mitochondrial membrane. For abbreviations, see Scheme 1.

Table 1. Summary of metabolic profiles

Eqn. no. in text	P/O ratio for NADH	Glucose C to: CO_2 (%)	Glucose C to: Fatty acid (%)	NADPH from pentose pathway (%)	NADPH from malate cycle (%)	Excess ATP per C_2 unit
1	3	39	58	23	74	0.18
2	3	48	48	100	0	5.5
3	2.8	39	58	25	72	0.14
4	2	40	56	36	61	0
5	2.8	41	56	38	60	0.98
6	2	41	55	37	63	0.18
7	3	38	58	21	77	0

the efficiency of conversion of glucose carbon into TG is lowered (Table 1), with the stoichiometry:

$$16.5\,\text{Glucose} + 132(\text{ADP}+\text{P}_i) \rightarrow 1\,\text{TG} + 48\,\text{CO}_2 + 132\,\text{ATP} \quad (2)$$

In addition, the excess ATP production is increased to 5.5 per acetyl group, close to the value of 5 produced from 16 glucose calculated by Flatt (1970) for these conditions. The extra half-glucose used in our model arises from the oxidation of a triose unit via pyruvate and the tricarboxylic acid cycle to balance the cytoplasmic $NAD^+/NADH$.

Does this excess ATP production arise from assumptions in the model, or is it a real effect, with the adipocyte finding uses for the ATP in other metabolic functions? Certainly, if the model is set to esterify non-esterified fatty acids in addition to those synthesized, then the ATP utilization balances its production. However, Flatt (1970) calculated the adipocyte's ATP requirement for cell maintenance from endogenous oxygen consumption and concluded that it could be entirely met by lipogenesis from glucose and that it would thereby limit the maximum rate of lipogenesis. Possible support for this claim comes from the observation that the brown adipose tissue of cold-acclimatized rats, which contains partially uncoupled mitochondria (Nicholls, 1974), is capable of higher rates of fatty acid synthesis from glucose than is white adipose tissue (McCormack & Denton, 1977; Trayhurn, 1979). Nevertheless, the consequences of changes in the assumptions in the model have been examined to determine how robust is the prediction of excess ATP synthesis. Firstly, the classical P/O ratios of 3 and 2 are probably overestimates. By using values of 2.8 and 1.9 [the former is the phenomenological stoichiometry calculated by Stucki (1980)], the difference in the result for minimum glucose utilization (cf. eqn. 1 and Scheme 2) is small:

$$13.89\,\mathrm{Glucose} + 3.35(\mathrm{ADP}+\mathrm{P_i}) \rightarrow 1\,\mathrm{TG} + 32.34\,\mathrm{CO_2} + 3.35\,\mathrm{ATP} \quad (3)$$

38% of the glucose flux goes via the pentose phosphate pathway. The use of P/O ratios of 2 and 1.3 (Hinkle & Yu, 1979) gives:

$$14.27\,\mathrm{Glucose} \rightarrow 1\,\mathrm{TG} + 34.62\,\mathrm{CO_2} \quad (4)$$

53% of the glucose flux goes via the pentose phosphate pathway, and there is no excess production of ATP. Other features of these solutions are shown in Table 1. The effects of the different P/O ratios are greater when the production of NADPH via the pentose phosphate pathway is maximized; the two results are:

$$14.33\,\mathrm{Glucose} + 23.7(\mathrm{ADP}+\mathrm{P_i}) \rightarrow 1\,\mathrm{TG} + 35\,\mathrm{CO_2} + 23.7\,\mathrm{ATP} \quad (5)$$

$$14.42\,\mathrm{Glucose} + 4.3(\mathrm{ADP}+\mathrm{P_i}) \rightarrow 1\,\mathrm{TG} + 35.52\,\mathrm{CO_2} + 4.3\,\mathrm{ATP} \quad (6)$$

The pattern of pathways is similar to that shown in Scheme 2, with fluxes through the pentose phosphate pathway of 56% and 59% of the glucose utilization respectively.

A second posssible cause of the excess ATP production might have been the need to balance mitochondrial phosphate by returning to the cytoplasm the excess that enters in exchange for malate (Scheme 2). If the coupling between the anion-transport processes were not complete, then the model would be too prescriptive. A formal way of representing relaxation of the coupling in the model is to incorporate a reaction allowing export of phosphate from the mitochondrion to the cytoplasm. Under these circumstances, the maximum efficiency of conversion of glucose is:

$$13.73\,\mathrm{Glucose} \rightarrow 1\,\mathrm{TG} + 31.38\,\mathrm{CO_2} \quad (7)$$

when the conventional P/O ratios are used. However, the predicted pattern of metabolism changes completely if P/O ratios of 2.8 and 1.9 are used; cytoplasmic ATP is supplied entirely by anaerobic glycolysis and lactate production, with very little pentose-pathway flux. This rather unrealistic result presumably reflects some difficulty in achieving exact stoichiometric balance with non-integral P/O ratios rather than an intrinsic effect of lower P/O ratios, since at values of 2 and 1.3, the result is the same as given in eqn. (4).

DISCUSSION

Any comparisons with experimental results cannot be exact, since there are likely to be variable levels of TG hydrolysis and esterification of non-esterified fatty acids in adipose tissue or adipocytes, as well as metabolism for cell maintenance. Nevertheless, it is striking that the patterns predicted do not involve lactate formation, which seems always to accompany other pathways of glucose utilization in experimental studies. The model can be constrained to predict the pathways followed when any arbitrary amount of esterification, lipolysis and lactate production is occurring, but there seems no requirement that lactate must be made. This would support the view that lactate is formed when the insulin-stimulated rate of entry of glucose to the tissue exceeds the capacity of the lipogenesis pathway. Apart from this, the pattern of Scheme 2 is similar to that suggested by Martin & Denton (1970); that is, there is little tricarboxylic-acid-cycle flux relative to citrate synthase activity, and both NADP-linked malate dehydrogenase and cytoplasmic isocitrate dehydrogenase contribute to the generation of cytoplasmic NADPH. The contribution of the pentose pathway to the NADPH requirement is here predicted to be lower than the experimental estimates unless the contribution of this pathway is maximized at the expense of the efficiency of conversion of glucose into TG.

These examples show that the requirements of balance of synthesis and degradation of each intermediate and coenzyme at the steady state can be powerful constraints on the relative rates of pathways, even in relatively large systems of reactions. Further, linear programming is a valuable method for rapidly calculating the consequences of changes in assumptions in the model. It would also be possible to build in limitations on the flux allowed through individual steps to accommodate information on maximal enzyme activities. The model used here could also be expanded to check that charge movements across the mitochondrial membrane were at steady state, if sufficient information were available about the elemental processes.

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